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FILE 'HOME' ENTERED AT 18:34:21 ON 30 MAY 2001

=> file medline biosis biotechno

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
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FILE 'MEDLINE' ENTERED AT 18:34:32 ON 30 MAY 2001

FILE 'BIOSIS' ENTERED AT 18:34:32 ON 30 MAY 2001

FILE 'BIOTECHNO' ENTERED AT 18:34:32 ON 30 MAY 2001

=> s bacteriorhodopsin or halorhodopsin or phoborhodopsin or (sensory rhodopsin)

L1 6702 BACTERIORHODOPSIN OR HALORHODOPSIN OR PHOBORHODOPSIN OR (SENSORY RHODOPSIN)

=> s l1 and(fused or fusion or chimer?)

L2 143 L1 AND(FUSED OR FUSION OR CHIMER?)

=> s l2 and py >1998

L3 28 L2 AND PY >1998

=> dup rem l3

PROCESSING COMPLETED FOR L3
L4 15 DUP REM L3 (13 DUPLICATES REMOVED)

=> d ibib abs 1-15

L4 ANSWER 1 OF 15 MEDLINE
ACCESSION NUMBER: 2001249722 MEDLINE
DOCUMENT NUMBER: 21226867 PubMed ID: 11327767
TITLE: Electron crystallographic analysis of two-dimensional crystals of **sensory rhodopsin** II: a 6.9 A projection structure.
AUTHOR: Kunji E R; Spudich E N; Grisshammer R; Henderson R; Spudich J L
CORPORATE SOURCE: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England.
CONTRACT NUMBER: R01GM27750 (NIGMS)
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, **(2001 Apr 27)** 308 (2) 279-93.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105

ENTRY DATE: Entered STN: 20010529
Last Updated on STN: 20010529
Entered PubMed: 20010430
Entered Medline: 20010524

AB Sensory rhodopsins, phototaxis receptors in Haloarchaea, were purified and reconstituted into halobacterial lipids to form photoactive two-dimensional crystals. Images of vitreous ice-embedded, flattened, tubular crystals of **sensory rhodopsin** II (SRII) of Natronobacterium pharaonis were recorded using a field emission gun electron cryo-microscope. Fourier components for the SRII structure were determined either from the separated image transforms from single layers that formed each side of flattened tubes, or by a deconvolution procedure when two layers were stacked in register so that they generated a single crystal lattice by superposition. Most micrographs showed significant diffraction to 6.9 A after computer processing, and the results provide the first intermediate- resolution information obtained for an archaeal **sensory rhodopsin**. The projection structure of SRII indicates that the helix positions match the seven-helix arrangement of the archaeal transport rhodopsins rather than that of the eukaryotic visual pigments. The structural similarity of SRII to the transport rhodopsins supports models in which the transport and signalling mechanisms of archaeal rhodopsins derive from the same retinal-driven changes in protein conformation. Copyright 2001 Academic Press.

L4 ANSWER 2 OF 15 MEDLINE
ACCESSION NUMBER: 2000412955 MEDLINE
DOCUMENT NUMBER: 20390033 PubMed ID: 10807928
TITLE: Evidence for post-translational membrane insertion of the integral membrane protein bacterioopsin expressed in the heterologous halophilic archaeon Haloferax volcanii.
AUTHOR: Ortenberg R; Mevarech M
CORPORATE SOURCE: Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(2000 Jul 28)** 275 (30) 22839-46.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000907
Last Updated on STN: 20000907
Entered Medline: 20000831

AB The gene coding for the integral membrane protein bacterioopsin (Bop), that is composed of seven transmembrane helices, was expressed in the halophilic archaeon Haloferax volcanii as a **fusion** protein with the halobacterial enzyme dihydrofolate reductase and with the cellulose binding domain of Clostridium thermocellum cellulosome. In each case, bacterioopsin was present both in the membrane and in the cytoplasmic fractions. Pulse-chase labeling experiments showed that the **fusion** protein in the cytoplasmic fraction is the precursor of the membrane-bound species. Bacterioopsin mutants that lack the seventh helix (BopDelta7) were found to accumulate only in the cytoplasmic fraction, whereas bacterioopsin mutants that lack either helices four and five (BopDelta4-5), or helices one and two (BopDelta1-2), were found in the cytoplasmic as well as in the membrane fractions. The seventh helix, when expressed alone, could target in trans the insertion of a separately expressed bacterioopsin mutant protein that has only the first six helices. These results support a model in which bacterioopsin is produced in H. volcanii as a soluble protein and in which its insertion into the membrane occurs post-translationally. According to this model, membrane insertion is directed by the seventh helix.

L4 ANSWER 3 OF 15 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001025171 MEDLINE

DOCUMENT NUMBER: 20442[redacted] PubMed ID: 10984603
TITLE: Proton transport by sensory rhodopsins its modulation by transducer-binding.
AUTHOR: Sasaki J; Spudich J L
CORPORATE SOURCE: Department of Space and Earth Science, Osaka University, Osaka 560-0043, Japan.
CONTRACT NUMBER: RO1-GM27750 (NIGMS)
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, **(2000 Aug 30)** 1460 (1) 230-9. Ref: 60
Journal code: A0W. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered PubMed: 20001103
Entered Medline: 20001115

AB The study of light-induced proton transfers in the archaeal sensory rhodopsins (SR), phototaxis receptors in Halobacterium salinarum, has contributed important insights into their mechanism of signaling to their cognate transducer subunits in the signaling complex. Essential features of the **bacteriorhodopsin** (BR) pumping mechanism have been conserved in the evolution of the sensors, which carry out light-driven electrogenic proton transport when their transducers are removed. The interaction of SRI with its transducer blocks proton-conducting channels in the receptor thereby inhibiting its proton pumping, indicating that the pump machinery, rather than the transport activity itself, is functionally important for signaling. Analysis of SRII mutants has shown that the salt bridge between the protonated Schiff base and its counterion Asp73 constrains the receptor in its inactive conformation. Similarly, in BR, the corresponding salt bridge between the protonated Schiff base and Asp85 contributes to constraining the protein in a conformation in which its cytoplasmic channel is closed. Transducer **chimera** studies further indicate that the receptor conformational changes are transmitted from the sensors to their cognate transducers through transmembrane helix-helix interaction. These and other results reviewed here support a signaling mechanism in which tilting of helices on the cytoplasmic side (primarily outward tilting of helix F), similar to that which occurs in BR in its open cytoplasmic channel conformation, causes structural alterations in the transducer transmembrane helices.

L4 ANSWER 4 OF 15 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000284874 MEDLINE
DOCUMENT NUMBER: 20284874 PubMed ID: 10826641
TITLE: Distribution and stability of membrane proteins in lipid membranes on solid supports.
AUTHOR: Puu G; Artursson E; Gustafson I; Lundstrom M; Jass J
CORPORATE SOURCE: Defence Research Establishment, Umea, Sweden.. puu@ume.foa.se
SOURCE: BIOSENSORS AND BIOELECTRONICS, **(2000 Mar)** 15 (1-2) 31-41.
Journal code: AKA; 9001289. ISSN: 0956-5663.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000810
Last Updated on STN: 20000810
Entered Medline: 20000727

AB **Bacteriorhodopsin** and the nicotinic acetylcholine receptor were

biotinylated and reconstituted in lipidic membranes on silicon supports by **fusion** with proteoliposomes. The presence and distribution of the proteins were studied by binding with streptavidin. Radio-labelled streptavidin was employed for quantifying the amounts of protein remaining in the supported membranes after storage in buffer. The proteins within the membranes remained bound to the surface for weeks. The biological activity of reconstituted unlabelled receptor upon storage showed stability in membranes formed on silicon supports and a reduced stability when formed onto lipid monolayer covered supports. Atomic force microscopy studies on preparations in liquid showed bilayer structures but also attached, partly **fused** liposomes and membrane particles. In air, the surface was smoother and contained less of liposomes and more of stacked lipid layers. Preparations labelled with streptavidin conjugated to colloidal gold and imaged in air showed the proteins individually distributed, with no protein-rich patches or protein aggregates.

L4 ANSWER 5 OF 15 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999367487 MEDLINE
DOCUMENT NUMBER: 99367487 PubMed ID: 10438533
TITLE: Bacterioopsin-triggered retinal biosynthesis is inhibited by **bacteriorhodopsin** formation in Halobacterium salinarium.
AUTHOR: Deshpande A; Sonar S
CORPORATE SOURCE: Protein Engineering Laboratory, Biotechnology Centre, Indian Institute of Technology, Powai, Mumbai 400 076, India.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1999 Aug 13)** 274 (33) 23535-40.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990913
Last Updated on STN: 19990913
Entered Medline: 19990901

AB Factors regulating retinal biosynthesis in halobacteria are not clearly understood. In halobacteria, events leading to the biosynthesis of **bacteriorhodopsin** have been proposed to participate in stringent regulation of retinal biosynthesis. The present study describes a novel approach of in vivo introductions of mRNA and membrane proteins via liposome **fusion** to test their role in cellular metabolism. Both the bacterioopsin-encoding mRNA and the liposome-encapsulated bacterioopsin (apoprotein) are independently introduced in spheroplasts of the purple membrane-negative strain Halobacterium salinarium that initially contain neither bacterioopsin nor retinal. Isoprenoid analyses of these cells indicate that the expression/presence of bacterioopsin triggers retinal biosynthesis from lycopene, and its subsequent binding to opsin generates **bacteriorhodopsin**. When **bacteriorhodopsin** and excess retinal were independently introduced into spheroplasts of purple membrane-negative cells, the introduction of **bacteriorhodopsin** resulted in an accumulation of lycopene, indicating an inhibition of retinal biosynthesis. These results provide direct evidence that the formation of bacterioopsin acts as a trigger for lycopene conversion to beta-carotene in retinal biosynthesis. The trigger for this event does not lie with either transcription or translation of the bop gene. It is clearly associated with the folded and the membrane-integrated state of bacterioopsin. On the other hand, the trigger signaling inhibition of retinal biosynthesis does not lie with the presence of excess retinal but with the correctly folded, retinal-bound form, **bacteriorhodopsin**.

L4 ANSWER 6 OF 15 MEDLI

ACCESSION NUMBER: 200003[illegible]6 MEDLINE
DOCUMENT NUMBER: 20031536 PubMed ID: 10563824
TITLE: Probing the folding and unfolding of wild-type and mutant forms of **bacteriorhodopsin** in micellar solutions: evaluation of reversible unfolding conditions.
AUTHOR: Chen G Q; Gouaux E
CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics, Columbia University, New York 10032, USA.
SOURCE: BIOCHEMISTRY, **(1999 Nov 16)** 38 (46) 15380-7.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991220

AB Wild-type and mutant forms of **bacteriorhodopsin** (sbR) from Halobacterium salinarium, produced by Escherichia coli overexpression of a synthetic gene, were reversibly unfolded in 1, 2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 3-[(3-cholamidopropyl)dimethylamino]-2-hydroxyl-1-propane (CHAPSO), and sodium dodecyl sulfate (SDS) mixed micelles. To study the effect on protein stability by substitutions on the hydrophobic surface with polar residues, the unfolding behavior of a G113Q, G116Q mutant [sbR(Q2)] was compared to the wild-type sbR [sbR(WT)]. sbR(Q2) was more sensitive to SDS-induced unfolding than sbR(WT) under equilibrium conditions, and kinetic experiments showed that sbR(Q2) was more sensitive to acid-induced denaturation and thermal unfolding than sbR(WT). Since the mutations in sbR(Q2) were on the detergent-embedded hydrophobic surface of sbR, protein destabilization by these mutations supports the concept that the membrane-embedded segments are important for the stability of sbR. Our experiments provide the basis for studying the thermodynamic stability of sbR by evaluating reversible folding and unfolding conditions in DMPC/CHAPSO/SDS mixed micelles.

L4 ANSWER 7 OF 15 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 1999322403 MEDLINE
DOCUMENT NUMBER: 99322403 PubMed ID: 10393291
TITLE: Specific lipid-protein interactions in a novel honeycomb lattice structure of **bacteriorhodopsin**.
AUTHOR: Sato H; Takeda K; Tani K; Hino T; Okada T; Nakasako M; Kamiya N; Kouyama T
CORPORATE SOURCE: Department of Physics, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan.
SOURCE: ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, **(1999 Jul)** 55 (Pt 7) 1251-6.
Journal code: C3C; 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1BM1
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990820
Last Updated on STN: 20000303
Entered Medline: 19990806

AB In the purple membrane of Halobacterium salinarium, **bacteriorhodopsin** trimers are arranged in a hexagonal lattice. When purple membrane sheets are incubated at high temperature with neutral detergent, membrane vesicularization takes place, yielding inside-out

vesicles with a diam[illegible]r of 50 nm. The vesicular [illegible]tru[illegible]re becomes unstable at low temp[illegible]ture, where successive **fusion** [illegible] the vesicles yields a crystal which is composed of stacked planar membranes. X-ray crystallographic analysis reveals that the **bacteriorhodopsin** trimers are arranged in a honeycomb lattice in each membrane layer and that neighbouring membranes orient in opposite directions. The native structure of the trimeric unit is preserved in the honeycomb lattice, irrespective of alterations in the in-plane orientation of the trimer. One phospholipid tightly bound to a crevice between monomers in the trimeric unit is suggested to act as a glue in the formation of the trimer.

L4 ANSWER 8 OF 15 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999128327 MEDLINE
DOCUMENT NUMBER: 99128327 PubMed ID: 9927658
TITLE: The specificity of interaction of archaeal transducers with their cognate sensory rhodopsins is determined by their transmembrane helices.
AUTHOR: Zhang X N; Zhu J; Spudich J L
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, The University of Texas Medical School, Houston, TX 77030, USA.
CONTRACT NUMBER: RO1-GM27750 (NIGMS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1999 Feb 2)** 96 (3) 857-62.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990305

AB **Chimeras** of the Halobacterium salinarum transducers HtrI and HtrII were constructed to study the structural determinants for their specific interaction with the phototaxis receptors sensory rhodopsins I and II (SRI and SRII), respectively. Interaction of receptors and transducers was assessed by two criteria: phototaxis responses by the cells and transducer-modulation of receptor photochemical reaction kinetics in membranes. Coexpression of HtrI with SRII or HtrII with SRI did not result in interaction by either criterion. Each receptor was coexpressed with **chimeric** transducers in which various domains of the two transducers were interchanged. The results show that the presence of the two transmembrane helices of HtrI in a **chimera** is necessary and sufficient for functional transducer complexation with SRI, i.e., for wild-type SRI photoreactions and attractant and 2-photon repellent phototaxis responses. Additionally, a previously demonstrated chaperone-like facilitation of SRI folding or stability by HtrI was shown to depend only on the two transmembrane helices of HtrI in **chimeric** transducers. Similarly, the two transmembrane helices of HtrII specify interaction with the repellent receptor SRII according to motility analysis and laser-flash spectroscopy. The results support a model in which the membrane domains of the receptor/transducer complexes, consisting of the seven helices of the receptor interacting with the four-helix bundle of the transducer dimer, produce SRI- and SRII-specific signals to the flagellar motor by means of interchangeable cytoplasmic domains.

L4 ANSWER 9 OF 15 BIOTECHNO
ACCESSION NUMBER: 1999:29362451 BIOTECHNO
TITLE: Evolutionary relationship between K.sup.+ channels and symporters
AUTHOR: Durell S.R.; Hao Y.; Nakamura T.; Bakker E.P.; Guy H.R.
CORPORATE SOURCE: Dr. H.R. Guy, Lab. of Exp./Computational Biol.,

tional Cancer Institute, [redacted] Institutes of alth, 12 South Drive, Bethesda[redacted]D 20892-5677, United States.
E-mail: guy@guy.nci.nih.gov
SOURCE: Biophysical Journal, (**1999**), 77/2 (775-788), 63 reference(s)
CODEN: BIOJAU ISSN: 0006-3495
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1999:29362451 BIOTECHNO.
AB The hypothesis is presented that at least four families of putative K.sup.+ symporter proteins, Trk and KtrAB from prokaryotes, Trk1,2 from fungi, and HKT1 from wheat, evolved from bacterial K.sup.+ channel proteins. Details of this hypothesis are organized around the recently determined crystal structure of a bacterial K.sup.+ channel: i.e., KcsA from Streptomyces lividans. Each of the four identical subunits of this channel has two fully transmembrane helices (designated M1 and M2), plus an intervening hairpin segment that determines the ion selectivity (designated P). The symporter sequences appear to contain four sequential M1-P-M2 motifs (MPM), which are likely to have arisen from gene duplication and **fusion** of the single MPM motif of a bacterial K.sup.+ channel subunit. The homology of MPM motifs is supported by a statistical comparison of the numerical profiles derived from multiple sequence alignments formed for each protein family. Furthermore, these quantitative results indicate that the KtrAB family of symporters has remained closest to the single-MPM ancestor protein. Strong sequence evidence is also found for homology between the cytoplasmic C-terminus of numerous bacterial K.sup.+ channels and the cytoplasm-resident TrkA and KtrA subunits of the Trk and KtrAB symporters, which in turn are homologous to known dinucleotide-binding domains of other proteins. The case for homology between bacterial K.sup.+ channels and the four families of K.sup.+ symporters is further supported by the accompanying manuscript, in which the patterns of residue conservation are demonstrated to be similar to each other and consistent with the known 3D structure of the KcsA K.sup.+ channel.

L4 ANSWER 10 OF 15 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1999:29422783 BIOTECHNO
TITLE: Bioenergetics of the Archaea
AUTHOR: Schafer G.; Engelhard M.; Muller V.
CORPORATE SOURCE: G. Schafer, Institut fur Biochemie, Medizinische Universitat zu Lubeck, 23538 Lubeck, Germany.
E-mail: schaefer@biochem.mu-luebeck.de
SOURCE: Microbiology and Molecular Biology Reviews, (**1999**), 63/3 (570-620), 628 reference(s)
CODEN: MMBRF7 ISSN: 1092-2172
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1999:29422783 BIOTECHNO
AB In the late 1970s, on the basis of rRNA phylogeny, Archaea (archaebacteria) was identified as a distinct domain of life besides Bacteria (eubacteria) and Eucarya. Though forming a separate domain, archaea display an enormous diversity of lifestyles and metabolic capabilities. Many archaeal species are adapted to extreme environments with respect to salinity, temperatures around the boiling point of water, and/or extremely alkaline or acidic pH. This has posed the challenge of studying the molecular and mechanistic bases on which these organisms can cope with such adverse conditions. This review considers our cumulative knowledge on archaeal mechanisms of primary energy conservation, in relationship to those of bacteria and eucarya. Although the universal

principle of chemiosmotic energy conservation and homologs for Archaea, distinct features have been discovered with respect to novel ion-transducing, membrane-residing protein complexes and the use of novel cofactors in bioenergetics of methanogenesis. From aerobically respiring archaea, unusual electron-transporting supercomplexes could be isolated and functionally resolved, and a proposal on the organization of archaeal electron transport chains has been presented. The unique functions of archaeal rhodopsins as sensory systems and as proton or chloride pumps have been elucidated on the basis of recent structural information on the atomic scale. Whereas components of methanogenesis and of phototrophic energy transduction in halobacteria appear to be unique to archaea, respiratory complexes and the ATP synthase exhibit some **chimeric** features with respect to their evolutionary origin. Nevertheless, archaeal ATP synthases are to be considered distinct members of this family of secondary energy transducers. A major challenge to future investigations is the development of archaeal genetic transformation systems, in order to gain access to the regulation of bioenergetic systems and to overproducers of archaeal membrane proteins as a prerequisite for their crystallization.

L4 ANSWER 11 OF 15 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 2000014702 MEDLINE
DOCUMENT NUMBER: 20014702 PubMed ID: 10545282
TITLE: Expression, purification, and structural characterization of the **bacteriorhodopsin**-aspartyl transcarbamylase **fusion** protein.
AUTHOR: Turner G J; Miercke L J; Mitra A K; Stroud R M; Betlach M C; Winter-Vann A
CORPORATE SOURCE: Department of Physiology & Biophysics, University of Miami School of Medicine, Miami, Florida, 33101, USA.. gturner@chroma.med.miami.edu
CONTRACT NUMBER: GM14053 (NIGMS)
GM31785 (NIGMS)
GM32079 (NIGMS)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, **(1999 Nov)** 17 (2) 324-38.
Journal code: BJV; 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000112

AB We are testing a strategy for creating three-dimensional crystals of integral membrane proteins which involves the addition of a large soluble domain to the membrane protein to provide crystallization contacts. As a test of this strategy we designed a **fusion** between the membrane protein **bacteriorhodopsin** (BR) and the catalytic subunit of aspartyl transcarbamylase from Escherichia coli. The **fusion** protein (designated BRAT) was initially expressed in E. coli at 51 mg/liter of culture, to yield active aspartyl transcarbamylase and an unfolded bacterio-opsin (BO) component. In Halobacterium salinarum, BRAT was expressed at a yield of 7 mg/liter of culture and formed a high-density purple membrane. The visible absorption properties of BRAT were indistinguishable from those of BR, demonstrating that the **fusion** with aspartyl transcarbamylase had no effect on BR structure. Electron microscopy of BRAT membrane sheets showed that the **fusion** protein was trimeric and organized in a two-dimensional crystalline lattice similar to that in the BR purple membrane. Following solubilization and size-exclusion purification in sodium dodecyl sulfate, the BO portion of the **fusion** was quantitatively refolded in tetradecyl maltoside (TDM). Ultracentrifugation demonstrated that BR and BRAT-TDM mixed micelles had molecular masses of 138 and 162 kDa, respectively, with a stoichiometry of one protein per micelle. High TDM

concentrations (>20 [redacted] were required to maintain BRA[redacted] olubility, hindering three-dime[redacted]onal crystallization trials. W[redacted]ave demonstrated that BR can functionally accommodate massive C-terminal fusions and that these fusions may be expressed in quantities required for structural investigation in H. salinarum.

L4 ANSWER 12 OF 15 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000014701 MEDLINE
DOCUMENT NUMBER: 20014701 PubMed ID: 10545281
TITLE: Heterologous gene expression in a membrane-protein-specific system.
AUTHOR: Turner G J; Reusch R; Winter-Vann A M; Martinez L; Betlach M C
CORPORATE SOURCE: Department of Physiology & Biophysics, University of Miami School of Medicine, Miami, Florida, 33101, USA.. gturner@chroma.med.miami.edu
CONTRACT NUMBER: GM14053 (NIGMS)
GM31785 (NIGMS)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, **(1999 Nov)** 17 (2) 312-23.
Journal code: BJV; 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J01671; GENBANK-M21410; GENBANK-M24335; GENBANK-X03010; GENBANK-X13530; GENBANK-X15263
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000112

AB We have constructed an expression system for heterologous proteins which uses the molecular machinery responsible for the high level production of **bacteriorhodopsin** in Halobacterium salinarum. Cloning vectors were assembled that **fused** sequences of the bacterio-opsin gene (bop) to coding sequences of heterologous genes and generated DNA fragments with cloning sites that permitted transfer of **fused** genes into H. salinarum expression vectors. Gene fusions include: (i) carboxyl-terminal-tagged bacterio-opsin; (ii) a carboxyl-terminal **fusion** with the catalytic subunit of the Escherichia coli aspartate transcarbamylase; (iii) the human muscarinic receptor, subtype M1; (iv) the human serotonin receptor, type 5HT2c; and (v) the yeast alpha mating factor receptor, Ste2. Characterization of the expression of these fusions revealed that the bop gene coding region contains previously undescribed molecular determinants which are critical for high level expression. For example, introduction of immunogenic and purification tag sequences into the C-terminal coding region significantly decreased bop gene mRNA and protein accumulation. The **bacteriorhodopsin** -aspartate transcarbamylase **fusion** protein was expressed at 7 mg per liter of culture, demonstrating that E. coli codon usage bias did not limit the system's potential for high level expression. The work presented describes initial efforts in the development of a novel heterologous protein expression system, which may have unique advantages for producing multiple milligram quantities of membrane-associated proteins.
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L4 ANSWER 13 OF 15 MEDLINE
ACCESSION NUMBER: 1999126081 MEDLINE
DOCUMENT NUMBER: 99126081 PubMed ID: 9929001
TITLE: Purification of histidine tagged **bacteriorhodopsin** , pharaonis **halorhodopsin** and pharaonis **sensory rhodopsin** II functionally expressed in Escherichia coli.
AUTHOR: Hohenfeld I P; Wegener A A; Engelhard M

CORPORATE SOURCE: Max-P[redacted]ck-Institut fur molekulare Phy[redacted]logie, Dortmund, Germa[redacted]
SOURCE: FEBS LETTERS, **(1999 Jan 15)** 442 (2-3) 198-202.
Journal code: EUH; 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990301
Last Updated on STN: 19990301
Entered Medline: 19990216

AB **Bacteriorhodopsin** (BR) from Halobacterium salinarum as well as **halorhodopsin** (pHR) and **sensory rhodopsin** II (pSRII) from Natronobacterium pharaonis were functionally expressed in E. coli using the method of Shimono et al. IFEBS Lett. (1997) 420, 54-56]. The histidine tagged proteins were purified with yields up to 1.0 mg/l cell culture and characterized by ESI mass spectrometry and their photocycle. The pSRII and pHR photocycles were indistinguishable from the wild type proteins. The BR photocycle was considerably prolonged. pSOII is located in the cytoplasmic membrane and the C-terminus is oriented towards the cytoplasm as determined by immunogold labelling.

L4 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:102967 BIOSIS
DOCUMENT NUMBER: PREV200000102967
TITLE: Photoresponse discrimination of **bacteriorhodopsin** films to light stimuli of different frequencies.
AUTHOR(S): Wang, Guangyu; Lu, Tao; Hu, Kun-Sheng (1); Jiang, Long
CORPORATE SOURCE: (1) Academia Sinica, Institute of Biophysics, Beijing, 100101 China
SOURCE: Journal of Photochemistry and Photobiology B Biology, (**Sept. Oct., 1999**) Vol. 52, No. 1-3, pp. 86-91.
ISSN: 1011-1344.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The capability of a **bacteriorhodopsin**-based artificial photosensor to discriminate the adjacent light stimuli from a moving object relies on the intrinsic properties of the differential photoresponse of **bacteriorhodopsin** films, such as peak value, peak width and degree of distortion of the biphasic spikes, which are related not only to the photoelectric detection system, but also to the pH, temperature, light intensity and the method for depositing films. At higher temperature (>300degreeC), lower pH (< 8.0) or in a deposited thin film rather than a Langmuir-Blodgett film, the relaxation of photoresponse will be accelerated and the sharp photocurrent spike will decrease the pre-excitatory inhibition of the next stimulus and enhance the **fusion** frequency. The **fusion** of photoresponse at high stimulus frequencies depends primarily on the response rise time rather than its decay. Under proper conditions, the maximum stimulus frequency corresponding to distinguishable signals in a deposited thin film can be as high as 30-50 Hz, contrasted with 10 Hz in a Langmuir-Blodgett film.

L4 ANSWER 15 OF 15 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1999196667 MEDLINE
DOCUMENT NUMBER: 99196667 PubMed ID: 10094768
TITLE: Extraction method for analysis of detergent-solubilized **bacteriorhodopsin** and hydrophobic peptides by electrospray ionization mass spectrometry.
AUTHOR: Barnidge D R; Dratz E A; Jesaitis A J; Sunner J
CORPORATE SOURCE: Department of Chemistry and Biochemistry, Montana State University, 108 Gaines Hall, Bozeman, Montana 58717-3520, USA.
CONTRACT NUMBER: AI22735 (NIAID)
AI26711 (NIAID)

[redacted] (NEI)
SOURCE: ANAL[redacted]CAL BIOCHEMISTRY, **(1999 Apr 10)** [redacted]69 (1) 1-9.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 19990614
Entered Medline: 19990528

AB The analysis of integral membrane proteins or transmembrane peptides by electrospray ionization mass spectrometry (ESI-MS) is difficult since detergents, used to solubilize these hydrophobic proteins and peptides, severely suppress analyte ion formation. This problem has been addressed previously by precipitating the protein, removing the detergent, and resolubilizing the protein in a nonpolar solvent. Here, we demonstrate a method that avoids protein precipitation and resolubilization. Detergent-solubilized **bacteriorhodopsin** is extracted into a nonpolar solvent phase by adding a chloroform/methanol/water solvent mixture to the aqueous detergent solution. ESI mass spectra of the nonpolar, chloroform-rich phase were dominated by peaks due to bacterioopsin. Bacterioopsin precursors with partially cleaved leader sequences were seen in all mass spectra. Additional peaks were likely due to intact **bacteriorhodopsin**, i.e., bacterioopsin with the retinal prosthetic group attached, and to bacterioopsin associated with lipid molecules. A separation process that occurred in the **fused** -silica capillary leading to the electrospray tip was essential for obtaining ESI mass spectra of bacterioopsin. The extraction-into-chloroform procedure also worked well with hydrophobic, transmembrane-type peptides that were insoluble in other electrospray solvents, including 100% formic acid, and the method has application to transmembrane peptides formed from digests of integral membrane proteins.


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=> s bacteriorhodopsin and(fused or fusion or chimer?)

L5          139 BACTERIORHODOPSIN AND(FUSED OR FUSION OR CHIMER?)

=> d his


     (FILE 'HOME' ENTERED AT 18:34:21 ON 30 MAY 2001)

     FILE 'MEDLINE, BIOSIS, BIOTECHNO' ENTERED AT 18:34:32 ON 30 MAY 2001
L1          6702 S BACTERIORHODOPSIN OR HALORHODOPSIN OR PHOBORHODOPSIN OR
(SENS
L2           143 S L1 AND(FUSED OR FUSION OR CHIMER?)
L3            28 S L2 AND PY >1998
L4            15 DUP REM L3 (13 DUPLICATES REMOVED)
L5           139 S BACTERIORHODOPSIN AND(FUSED OR FUSION OR CHIMER?)

=> s l2 not l5

L6             4 L2 NOT L5

=> dup rem l6

PROCESSING COMPLETED FOR L6
L7             3 DUP REM L6 (1 DUPLICATE REMOVED)

=> d ibib abs 1-3


L7   ANSWER 1 OF 3  BIOSIS  COPYRIGHT 2001 BIOSIS
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ACCESSION NUMBER: 1999:[redacted]934 BIOSIS
DOCUMENT NUMBER: PREV1[redacted]00152934
TITLE: The specificity of interaction of archaeal transducers with
their cognate sensory rhodopsins is determined by their transmembrane helices.
AUTHOR(S): Zhang, Xue-Nong; Zhu, Jingya; Spudich, John L. (1)
CORPORATE SOURCE: (1) Dep. Microbiol. Mol. Genetics, Univ. Texas Med. Sch., Houston, TX 77030 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (Feb. 2, 1999) Vol. 96, No. 3, pp. 857-862.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English

AB **Chimeras** of the Halobacterium salinarum transducers HtrI and HtrII were constructed to study the structural determinants for their specific interaction with the phototaxis receptors sensory rhodopsins I and II (SRI and SRII), respectively. Interaction of receptors and transducers was assessed by two criteria: phototaxis responses by the cells and transducer-modulation of receptor photochemical reaction kinetics in membranes. Coexpression of HtrI with SRII or HtrII with SRI did not result in interaction by either criterion. Each receptor was coexpressed with **chimeric** transducers in which various domains of the two transducers were interchanged. The results show that the presence of the two transmembrane helices of HtrI in a **chimera** is necessary and sufficient for functional transducer complexation with SRI, i.e., for wild-type SRI photoreactions and attractant and 2-photon repellent phototaxis responses. Additionally, a previously demonstrated chaperone-like facilitation of SRI folding or stability by HtrI was shown to depend only on the two transmembrane helices of HtrI in **chimeric** transducers. Similarly, the two transmembrane helices of HtrII specify interaction with the repellent receptor SRII according to motility analysis and laser-flash spectroscopy. The results support a model in which the membrane domains of the receptor/transducer complexes, consisting of the seven helices of the receptor interacting with the four-helix bundle of the transducer dimer, produce SRI- and SRII-specific signals to the flagellar motor by means of interchangeable cytoplasmic domains.

L7 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 1993:232979 BIOSIS
DOCUMENT NUMBER: PREV199395124154
TITLE: Homologous overexpression of a light-driven anion pump in an archaebacterium.
AUTHOR(S): Heymann, Juergen A. W.; Havelka, Wendy A.; Oesterhelt, Dieter (1)
CORPORATE SOURCE: (1) Max-Planck-Inst. fuer Biochemie, D-8033 Martinsried Germany
SOURCE: Molecular Microbiology, (1993) Vol. 7, No. 4, pp. 623-630.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The retinal protein **halorhodopsin** (HR), a light-driven chloride pump from Halobacterium halobium, was homologously overexpressed in this archaebacterium. Two DNA expression system differing in their promoter region were investigated. The haloopsin, hop, promoter coupled to the hop gene gave an increased level of HR synthesis. However, the extent of expression was driven by the copy number of the shuttle vector and did not reach the magnitude of the bacterio-opsin, bop, promoter system. Employing a gene **fusion** approach, the promoter for the bop gene was used to drive expression of the hope gene. A shuttle vector containing a bop-hop-cartridge was transformed into a HR-deficient strain and blueish-coloured transformants were obtained. The bop promoter expressed HR to an extent where a specific membrane fraction resembled the crystalline purple membrane of BR in terms of the lipid to protein ratio.

HR could, therefore, easily isolated in a natural membrane-bound state.

This allows for direct use in biophysical studies without the application of detergents. This was the first successful overexpression of a 7-helical transmembrane protein and may be extended to other proteins of this family.

L7 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:131834 BIOSIS
DOCUMENT NUMBER: PREV199344062834
TITLE: Overexpression of **halorhodopsin**: New perspectives for structure-function studies.
AUTHOR(S): Heymann, Juergen A. W.; Havelka, W. A.; Oesterhelt, D.
CORPORATE SOURCE: Max-Planck Inst. Biochem., Am Klopferspitz 18A, 8033 Martinsried Germany
SOURCE: Rigaud, J.-L. [Editor]. Colloque INSERM, (1992) Vol. 221, pp. 329-332. INSERM Colloquium; Structures and functions of retinal proteins. Colloque INSERM; Structures et fonctions des retino-proteines.
Publisher: Editions INSERM (Institut National de la Sante et de la Recherche Medicale) 101 rue de Tolbiac, F-75013 Paris, France.
Meeting Info.: Vth International Conference on Retinal Proteins Dourdan, France June 28-July 3, 1992
ISSN: 0768-3154. ISBN: 2-85598-509-9, 0-86196-355-5.
DOCUMENT TYPE: Article
LANGUAGE: English

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L4 15 DUP REM L3 (13 DUPLICATES REMOVED)
L5 139 S BACTERIORHODOPSIN AND(FUSED OR FUSION OR CHIMER?)
L6 4 S L2 NOT L5
L7 3 DUP REM L6 (1 DUPLICATE REMOVED)